REMARKS

Applicants' invention focuses on purified nucleic acid sequences encoding AGE-1 polypeptides, and methods that exploit these *age-1* nucleic acids for the identification of compounds that decrease AGE-1 expression or activity.

Support for the Amendments

The amendments to claims 12, 15, 16, and 29 find support throughout the specification, for example, as follows: claim 12, pages 28 and 29; claim 15, page 19, lines 14-33, and page 31, lines 9-27; claim 16, page 32, lines 14-21, and page 35, lines 23-26; and claim 29, Figure 6.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 15 and 18-20 stand rejected, under 35 U.S.C. § 112, first paragraph, based on the assertion that the claims contain subject matter that does not satisfy the written description requirement, as set forth in the PTO interim guidelines. As applied to the current claims, this rejection is respectfully traversed.

With respect to claim 15 and dependent claims 18-20, the Office states that the specification discloses only SEQ ID NO:1, the amino acid sequence of an AGE-1 polypeptide, and SEQ ID NO:2, the cDNA encoding SEQ ID NO:1. The Office states that the specification fails to disclose the *age-1* promoter and therefore fails to satisfy the written description requirement. Applicants respectfully disagree. However, to expedite

prosecution, Applicants have amended claim 15 to specify that a nematode cell expressing endogenous *age-1* is utilized in the claimed assay. Clearly, such an assay does not require isolation or identification of the AGE-1 promoter sequence, and this rejection may be withdrawn.

In addition, claims 8, 10-13, 29, and 30 stand rejected as lacking enablement. This rejection turns on the assertion that there is nothing in the specification or prior art to show that the AGE-1 polypeptide functions as a kinase. The Office states that, in the absence of such a functional assay, an artisan would not know how to use the claimed polynucleotides, expression vectors, and other claimed materials. This rejection is respectfully traversed.

Applicants submit that there is no reason to believe, and no documentary evidence has been provided by the Office to suggest, that AGE-1 polypeptides would fail to possess PI 3-kinase activity. To the contrary, a great deal of evidence has been provided by Applicants indicating that AGE-1 does indeed function as a PI 3-kinase. First, as previously pointed out by Applicants, AGE-1 proteins possess structural motifs characteristic of the p110 family of kinases, such as a lipid kinase domain and p85-binding motifs. Applicants agree that the presence of these domains is not dispositive of the issue of protein function, but such domains are clearly consistent with AGE-1 having protein kinase activity.

Moreover, although AGE-1 represents a divergent member of the p110 family, it nonetheless possesses significant homology with other p110 kinases. The Office's attention is directed to page 22, lines 18-20, of the specification, where Applicants show

that the random probability of alignment of AGE-1 with p110 kinases is exceedingly low. This statistically significant homology alone is not dispositive of AGE-1's role as a kinase, but is again strongly indicative of AGE-1 having such kinase activity.

As yet another line of evidence indicating that *age-1* would be considered by those of skill in the art to encode a PI 3-kinase, Applicants further direct the Office's attention to the publication by Morris *et al.*, submitted in connection with the previous reply. This publication by the inventors states that *age-1* encodes a homologue of mammalian phosphatidylinositol-3-OH kinase catalytic subunits. These results were peer-reviewed by top scientists and published in the prestigious journal, *Nature*. The fact that researchers chosen to review articles for *Nature* were convinced that *age-1* nucleic acids encode PI 3-kinase proteins demonstrates that one skilled in the art believes that AGE-1 is a PI 3-kinase.

In addition, Applicants have previously submitted another publication by Babar *et al.* (Exhibit B of the previous reply). This publication, authored by a group distinct from Applicants, provides yet further evidence that AGE-1 polypeptides are PI 3-kinases. In this reference, the authors treated *C. elegans* with a known chemical inhibitor of mammalian PI 3-kinases, a chemical termed LY294002. This treatment mimicked the effects of AGE-1 mutations (pages 516-517), as measured by dauer formation, thermotolerance, and life span. Once more, this result is completely consistent with AGE-1 functioning as a PI 3-kinase.

Despite this extremely strong and entirely consistent evidence indicating AGE-1's role as a PI 3-kinase, the Office has maintained the enablement rejection stating:

> It is interesting that even 5 years after the publication of the Morris paper, which teaches the amino acid sequence of AGE-1, there is no publication or evidence of record regarding the activity of the purified protein or recombinantly expressed protein. This clearly indicates difficulties in assaying the activity of the protein and expression of the protein *in vitro*. Therefore, the issue raised in the enablement rejection is a valid issue... (page 7, lines 24-30)

Applicants strongly disagree with the Office's conclusion, and offer an alternative explanation. Given the overwhelming evidence in favor of the AGE-1 protein functioning as a PI 3-kinase, one skilled in the art would not be motivated to express the protein *in vitro* and assay its activity. Applicants submit that the fact that no skilled artisan has bothered to test AGE-1's kinase activity *in vitro* is equally consistent with the position that skilled artisans accept that *age-1* encodes a PI 3-kinase protein and see no reason to carry out redundant experiments.

Finally, the Office expresses concern about the AGE-1 PI 3-kinase in nematodes carrying out the role of multiple mammalian proteins. The Office questions how an artisan could assay the function of a protein that has the activity of three mammalian kinase subunits. Again, Applicants point out that this concern is unwarranted. In fact, it is understood that, during the evolution of a diploid organism, a single gene, for example, a PI 3-kinase, may undergo duplication. This duplication may occur multiple times. Over the course of evolution, these duplicated genes may and typically do diverge. Thus, the highly evolved and complex mammalian genome might possess multiple genes, for example, multiple PI 3-kinase genes, fulfilling overlapping or slightly different roles, while the simple *C. elegans* genome might possess a single gene, for example, a single PI 3-kinase gene, that plays that same role. Nonetheless, the inherent activity of each gene's

encoded protein remains the same, that is, remains a PI 3-kinase. From this example, it is clear that *C. elegans age-1* need not fully possess the activity of three mammalian PI 3-kinase subunits, but rather that the multiple mammalian PI 3-kinase subunits may have diverged during the course of evolution from an original PI 3-kinase protein. Therefore, it is not necessary for an artisan to assay the function of a protein that has the activity of three proteins. It is only necessary for the skilled artisan to assay the activity of the one protein encoded by *age-1*, a PI 3-kinase, using the methods provided within the specification.

In summary, Applicants have provided overwhelming evidence that AGE-1 is a PI 3-kinase. Applicants have shown by characteristic structural motifs and statistically significant homology that *age-1* encodes a homolog of mammalian PI 3-kinases. As would be predicted on the basis of this homology, Applicants have also provided evidence that an inhibitor of mammalian PI 3-kinases mimics the effects of *age-1* mutations in *C. elegans*. Moreover, an article by the inventors was approved for publication in the prestigious journal, *Nature*, demonstrating that one skilled in the art would agree with the inventors that *age-1* encodes a PI 3-kinase. Applicants submit that strong evidence has been presented in Applicants' specification and in this reply for the function of AGE-1 as a PI 3-kinase. In contrast, no documentary evidence has been provided by the Office that would cause one to question Applicants' conclusion. Applicants request that such documentary evidence be provided or, in the absence of such evidence, that the enablement rejection be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 12, 15, 16, 18-20, and 29-30 stand further rejected, under 35 U.S.C. § 112, second paragraph, based on the assertion that certain claim terms are indefinite. This rejection has been met by the above claim amendments, and may be withdrawn.

Conclusion

Applicants submit that this case is in condition for allowance, and such action is respectfully requested. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Marked-up and clean versions of the amended claims are enclosed.

Also enclosed is a petition to extend the period for replying for three months, to and including December 7, 2001. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 7 December 2001

Karen L. Elbing, Ph.D.
Reg. No. 35,238

Clark & Elbing LLP
176 Federal Street
Boston, MA 02110-2214
Telephone: 617-428-0200
Facsimile: 617-428-7045

Marked-up Version of Amended Claims

12. (Twice Amended) A method of producing a recombinant AGE-1 polypeptide, said method comprising the steps of:

(a) providing a cell transformed with the DNA of claim 8 encoding an AGE-1 polypeptide, said DNA being expressed [positioned for expression] in the cell;

(b) culturing the transformed cell under conditions for expressing the DNA; and

(c) isolating the recombinant AGE-1 polypeptide.

15. (Twice Amended) A method of identifying an AGE-1 modulatory compound that is capable of decreasing the expression of an AGE-1 gene, said method comprising the steps of:

(a) providing a nematode cell expressing the endogenous AGE-1 DNA of claim 8 ,

(b) contacting said nematode cell with a candidate compound; and

(c) measuring AGE-1 gene expression in said nematode cell, a decrease in AGE-1 gene expression in said nematode cell following contact with said candidate compound, relative to AGE-1 gene expression in an untreated nematode cell, identifying said candidate compound as a compound that is capable of decreasing AGE-1 gene expression.

16. (Thrice Amended) A method of identifying an AGE-1 modulatory compound that is capable of decreasing AGE-1 PI 3-kinase activity, said method comprising the steps of:

(a) providing a cell expressing an AGE-1 polypeptide of claim 8;

(b) contacting the cell with a candidate compound; and

(c) measuring the PI 3-kinase activity of said cell, a decrease in AGE-1 PI 3-kinase activity of said cell following contact with the candidate compound, relative to AGE-1 PI 3-kinase activity in an untreated cell, identifying said candidate compound as a compound that is capable of decreasing AGE-1 PI 3-kinase activity.

29. (Twice Amended) The purified and isolated DNA of claim 8, wherein said polypeptide comprises at least 50% of the following amino acids of Figure 6 (SEQ ID NO: 1) at the indicated amino acid positions: amino acids Gly-32, Leu-73, His-78, Phe-81, Glu-109, Phe-114, Leu-123, Leu-125, Phe-129, Lys-181, Ser-208, Lys-211, Arg-321, Leu-325, Leu-351, Ser-355, Met-373, Leu-381, Leu-393, Thr-432, Tyr-451, Glu-475, Pro-507, Ile-514, Gly-518, Glu-530, Val-538, Leu-582, Tyr-606, Pro-643, Phe-665, Leu-744, Leu-745, Arg-762, Leu-789, Arg-794, Ala-827, Arg-829, Trp-835, Ser-842, Asn-905, Gly-917, Asp-975, Ile-990, Asp-1006, His-1020, Lys-1104, Thr-1105, Gly-1130, Phe-1140, and Lys-1144

Clean Version of All Pending Claims

8. A purified and isolated DNA which encodes an AGE-1 polypeptide having PI 3-kinase activity, said polypeptide having at least 95% amino acid sequence identity to the full length polypeptide of Figure 6 (SEQ ID NO: 1) and comprising a p85-binding domain and a lipid kinase domain.

10. A vector comprising the purified and isolated AGE-1 DNA of claim 8.

11. A cell comprising the purified and isolated AGE-1 DNA of claim 8.

12. (Twice Amended) A method of producing a recombinant AGE-1 polypeptide, said method comprising the steps of:

(a) providing a cell transformed with the DNA of claim 8 encoding an AGE-1 polypeptide, said DNA being expressed in the cell;

(b) culturing the transformed cell under conditions for expressing the DNA; and

(c) isolating the recombinant AGE-1 polypeptide.

13. A recombinant AGE-1 polypeptide produced according to the method of claim 12.

15. (Twice Amended) A method of identifying an AGE-1 modulatory compound that is capable of decreasing the expression of an AGE-1 gene, said method comprising the steps of:

(a) providing a nematode cell expressing the endogenous AGE-1 DNA of claim 8 ,

(b) contacting said nematode cell with a candidate compound; and

(c) measuring AGE-1 gene expression in said nematode cell, a decrease in AGE-1 gene expression in said nematode cell following contact with said candidate compound, relative to AGE-1 gene expression in an untreated nematode cell, identifying said candidate compound as a compound that is capable of decreasing AGE-1 gene expression.

16. (Thrice Amended) A method of identifying an AGE-1 modulatory compound that is capable of decreasing AGE-1 PI 3-kinase activity, said method comprising the steps of:

(a) providing a cell expressing an AGE-1 polypeptide of claim 8;

(b) contacting the cell with a candidate compound; and

(c) measuring the PI 3-kinase activity of said cell, a decrease in AGE-1 PI 3-kinase activity of said cell following contact with the candidate compound, relative to AGE-1 PI 3-kinase activity in an untreated cell, identifying said candidate compound as a compound that is capable of decreasing AGE-1 PI 3-kinase activity.

18. The method of claim 15 or 16, wherein said AGE-1 gene or AGE-1 polypeptide is from an animal.

19. The method of claim 15 or 16, wherein said method is carried out in a nematode

20. The method of claim 15 or 16, wherein said method involves assaying AGE-1 PI 3-kinase activity *in vitro*.

29. (Twice Amended) The purified and isolated DNA of claim 8, wherein said polypeptide comprises at least 50% of the following amino acids of Figure 6 (SEQ ID NO: 1) at the indicated amino acid positions: amino acids Gly-32, Leu-73, His-78, Phe-81, Glu-109, Phe-114, Leu-123, Leu-125, Phe-129, Lys-181, Ser-208, Lys-211, Arg-321, Leu-325, Leu-351, Ser-355, Met-373, Leu-381, Leu-393, Thr-432, Tyr-451, Glu-475, Pro-507, Ile-514, Gly-518, Glu-530, Val-538, Leu-582, Tyr-606, Pro-643, Phe-665, Leu-744, Leu-745, Arg-762, Leu-789, Arg-794, Ala-827, Arg-829, Trp-835, Ser-842, Asn-905, Gly-917, Asp-975, Ile-990, Asp-1006, His-1020, Lys-1104, Thr-1105, Gly-1130, Phe-1140, and Lys-1144

30. The purified and isolated DNA of claim 29, wherein said polypeptide comprises an identical amino acid in the equivalent position to Ala-827 of Figure 6 (SEQ ID NO: 1).